Reproduction in Domestic Animals

Reprod Dom Anim **46** (Suppl. 2), 31–34 (2011); doi: 10.1111/j.1439-0531.2011.01853.x
ISSN 0936-6768

Plenary Article

Plenary Contribution to International Conference on Boar Semen Preservation 2011. Genetic Selection for Freezability and its Controversy with Selection for Performance

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Contents

Little data are available in the literature regarding freezability of boar sperm or its relationship with other traits. Existing data suggest the trait would respond favourably to selection, and information is available from other species suggesting components that might have changed. Genetic parameters are estimated for boar sperm freezability including heritability and correlations with other production traits. Sperm freezability is an ideal candidate for marker assisted-selection or selection for favourable alleles.

Introduction

Artificial insemination in pigs is popular compared with other livestock species despite nearly all matings utilizing fresh, diluted semen rather than frozen because of the poor fertility resulting from post-thaw boar semen. In swine, frozen semen is used mostly for three purposes: conservation (long-term goal and high-value samples if used); international export (allows storage of semen while donor is quarantined at original location); research such as IVF (small samples and using the same ejaculate reduces experimental variation). In each of these settings, variation among boars has been observed and a genetic component is implied. This review discusses genetic considerations for boar sperm freezability.

Few data are published on the genetics of semen freezing. Breed and line differences are recognized (Woelders et al. 1996; Pizzi et al. 2005; Waterhouse et al. 2006). In two populations, amplified fragment length polymorphism markers were identified as being associated with 'good' and 'poor' freezers (Thurston et al. 2002; Fraser et al. 2008). Evaluation of markers in progeny of these boars or other populations to confirm associations with freezability have not been reported. Proportions of boars rated as 'good' or 'bad' freezers varies considerably across populations (Medrano et al. 2002; Thurston et al. 2002; Roca et al. 2006). The greatest amount of variation in post-thaw sperm quality is often the individual animal, and post-thaw assessments result in greater variation among boars than fresh comparisons (Larsson and Einarsson 1976; Medrano et al. 2002; Holt et al. 2005; Hernandez et al. 2006; Roca et al. 2006; Waterhouse et al. 2006).

Genetic Selection and Literature Estimates

Given the variation observed in post-thaw viability and the fact that breed and individual animal variation are common, many have asked whether it would respond to genetic selection. Response to selection is described mathematically according to equation 1.

$$\text{genetic change} = \text{heritability} \times \text{selection differential} \quad eqn1$$

Heritability is simply the proportion of observed phenotypic variation attributable to additive genetic variance. Selection differential is the difference between the mean of the population and the mean of those selected as parents of the next generation.

Heritabilities for most reproductive traits are low, indicating the majority of observed variation is non-genetic and the trait will not likely respond rapidly to selection. Selection for freezability in swine is not published. A series of reports was published in chickens selecting for duration of post-thaw fertility (e.g. needed frequency of insemination). The trait responded to selection as expected, and after five generations of selection, a realized heritability of 0.17 ± 0.05 was reported (Ansah and Buckland 1983). Duration of fertility increased an average of 0.29 days per generation between generations 3 and 8 (Ansah and Buckland 1982a).

Another question in the discussion of genetics of sperm freezability is the controversy of selection for freezability with other production traits. Two possible reasons to expect such a relationship are dilution of selection intensity by adding another trait and unfavourable correlations of freezability with other traits under selection. To understand the former refer to equation 1 for a single trait. Use of selection indices allows simultaneous selection for multiple traits as long as genetic and phenotypic correlations among traits of interest are known. When multiple traits are selected, intensity of selection for each trait is reduced compared with that possible if single trait selection was practiced. For the latter situation, a trait correlated with a trait

under selection will change in the population as a correlated response to selection. For example, selection to reduce backfat tends to reduce appetite, resulting in dramatic reductions in growth rate.

Selection for freezability is likely to alter sperm cell membranes or seminal plasma. Neither of these is obviously related to other important traits unless changes in carcass fat levels would affect lipid membranes in sperm. In the chicken selection experiment, correlated responses in other fertility traits were positive with no correlations with mature body weight or mature testis size (Yousif et al. 1984) and no correlation between fertility of fresh semen and frozen semen (Mitchell et al. 1977), although semen volume and number of sperm per ejaculate were reduced (Ansah et al. 1985). Improvements in duration of fertility may have been from one or more correlated responses, increased membrane permeability to glycerol as well as carrier-mediated substances, changes in membrane cholesterol and phospholipid ratios (Ansah and Buckland 1982a,b; respectively), changes in oxygen uptake (Scott et al. 1980) and changes in seminal plasma protein profiles (Bentley et al. 1984). Investigating differences in these specific parameters in 'good' or 'bad' freezing boars with currently available tools merits consideration.

Boar Freezability Genetic Parameters

To evaluate prospects for response to genetic selection, heritabilities and genetic correlations must be estimated. Because of the absence of such data in the literature, the following is provided. Genetic parameters were estimated from 920 ejaculates collected over 7 years from 254 individual boars from a four breed composite. Boars were maintained in an environmentally controlled barn at the USDA Meat Animal Research Center in Clay Center, NE. Semen collection and processing were as described previously (Guthrie and Welch 2005). Briefly, semen was collected by the gloved hand technique, concentration determined and then diluted 1 : 1 in BTS and allowed to cool to room temperature for 90 min. Extended semen was aliquoted 12×10^9 cells per 50-ml conical tube, brought to 16°C and shipped to the USDA BARC in MD. At BARC, samples were centrifuged and cells resuspended in lactose egg yolk cooling extender prior to use of glycerol-based freezing medium. Data were fitted to a model including fixed effects for month/season of collection, collection date and boar age as a covariate. An animal model with a second random effect of permanent environment was fitted. Genetic parameters are presented in Table 1 and show relatively low heritabilities, and heritability of live percentage is lower in thawed than in fresh semen, indicating a greater amount of observed variation owing to environment. Heritabilities of CASA traits are presented in Table 2 as well as the genetic correlations between the CASA traits on fresh and on frozen sperm. Genetic correlations are surprisingly high given reports indicating that fresh analyses are not predictive of thawed sperm quality (Hernandez et al. 2007b). Table 3 presents correlations between estimated breeding value (EBV) for weight (wt) and backfat at 154 day with %live cells in fresh and in frozen samples, mortality and %mortality. Statistically significant correlations are as expected, and there are no significant correlations of semen traits with either wt or backfat at 154 day. The correlation between 154 day wt and %mortality and

Table 1. Estimated variance components[a] for fresh (24 h) and frozen/thawed sperm traits including percentage cells alive in fresh and frozen/thawed samples, mortality (the difference between the two measures) and % mortality (mortality/fresh%live)

Trait	Additive genetic	Permanent environment	Error	Heritability (SE)[b]
Fresh%live	39.85	6.09	22.37	0.583 (0.147)
Thawed%live	23.15	0.00	78.22	0.228 (0.077)
Mortality	8.33	9.06	70.64	0.095 (0.063)
%mortality	10.99	4.70	84.51	0.110 (0.060)

[a]Variance components were estimated from a mixed model with repeated measures. Fixed effects were season, date and age (covariate) of boar at collection. Random effects fitted were animal (including the additive genetic relationship matrix), permanent environment and an error term.
[b]Heritability (standard error).

Table 2. Heritabilities (and standard errors) for fresh (24 h old) and frozen/thawed boar sperm for percentage live cells and CASA parameters (% motility, amplitude of lateral head displacement, beat cross frequency, track velocity and straight line velocity) plus genetic correlations between fresh and frozen/thawed heritabilities

Trait	Heritability fresh	Heritability thawed	Genetic correlation
Motility (%)	0.192 (0.045)	0.367 (0.098)	0.663 (0.133)
Per cent live	0.514 (0.108)[a]	0.193 (0.070)	0.824 (0.124)
ALH, (μm)	0.139 (0.038)	0.083 (0.059)	0.962 (0.227)
BCF (Hz)	0.057 (0.022)	0.281 (0.085)	0.465 (0.239)
VCL, (μm/s)	0.139 (0.039)	0.232 (0.089)	0.888 (0.126)
VSL (μm/s)	0.090 (0.030)	0.364 (0.082)	1.00 (0.082)

[a]Evaluated 24 h after collection.

	154 day backfat	154 day wt	Fresh%live	Thaw%live	Mortality
154 day wt	0.487 (< 0.0001)				
Fresh%live[a]	−0.010 (0.88)	0.052 (0.41)			
Thaw%live	0.044 (0.49)	0.106 (0.09)	0.590 (< 0.0001)		
Mortality	−0.064 (0.31)	−0.105 (0.09)	0.314 (0.0001)	−0.534 (< 0.0001)	
%mortality	−0.040 (0.53)	−0.058 (0.36)	−0.104 (0.10)	−0.706 (< 0.0001)	0.619 (< 0.0001)

[a]Evaluated 24 h after collection.

Table 3. Correlations (and p values) between estimated breeding values (EBV) for 154 day backfat, 154 day weight, percentage live cells in fresh (fresh%live) and in thawed (thaw%live) semen, absolute mortality (fresh%live−thaw%live) and mortality percentage (mortality/fresh%live). No significant correlations exist with production traits

%live in thawed samples approaches significance in a favourable direction.

Selection Strategies

Many male reproductive traits exhibit low heritability and have unfavourable relationships with production traits of progeny (see Safranski 2008 for review). Heritabilities for freezability presented in this paper are low to moderate but higher than for fresh semen traits and without unfavourable relationships with 154 day wt or backfat. This suggests that direct selection for freezability would be expected to be successful as was demonstrated in chickens. Incorporating selection for freezability into an index with other economically important traits requires confirmation of correlations between it and other traits and an economic value assigned to freezability.

Alternatives would be to practice indirect selection for components of freezability if screening for these parameters is easier than estimating freezability. This might include selection for increased membrane fluidity, oxygen uptake or other traits expected to be associated with freezability. Initially, it might be prudent to screen for variation in these traits in boars known to be 'good' or 'bad' freezers. Greatest practical application of these procedures would be realized if there is high correlation between their expression in somatic cells and that in sperm cells such that they could be determined early in life without the need to collect semen. Variation in seminal plasma cannot be ignored, especially given reports that addition of seminal plasma from 'good' freezing boars increased viability of frozen boar sperm (Hernandez et al. 2007a).

Sperm freezability is only expressed in one sex, only relatively late in life, and is not easy to measure, making it an ideal candidate for marker-assisted selection or direct selection on favourable alleles. Such an approach could be applied to tissues early in life (e.g. docked tails or ear notches) once appropriate tests are developed. This could be performed either by confirming strong associations of markers, likely population specific, or using gene chips to identify favourable genetic variants for component traits. The latter approach would require screening in large pedigreed populations and measuring sperm freezability in those populations.

Although little data exist in the literature for freezability, what exists does not indicate unfavourable relationship with other traits. Data presented in this paper, in fact, show non-significant but favourable relationships with growth. Genetic parameters should be confirmed in multiple populations, but data are encouraging that freezability could be improved through genetic selection without negative impact on growth and backfat thickness.

Acknowledgements

Authors acknowledge help from G Welch, A Kruger and USMARC Swine Operations staff.

Conflicts of interest

None declared.

References

Ansah GA, Buckland RB, 1982a: Genetic variation in fowl semen cholesterol and phospholipid levels and the relationship of these lipids with fertility of frozen-thawed and fresh semen. Poultry Sci **61,** 623–637.

Ansah GA, Buckland RB, 1982b: The uptake of glycerol, α-Aminoisobutyric acid and 2-deoxy-D-glucose by spermatozoa of a line of chickens selected for fertility of frozen-thawed semen and a control line. Theriogenology **17,** 401–408.

Ansah GA, Buckland RB, 1983: Eight generations of selection for duration of fertility of frozen-thawed semen in the chicken. Poultry Sci **62,** 1529–1538.

Ansah GA, Segura JC, Buckland RB, 1985: Semen production, sperm quality, and their heritabilities as influenced by selection for fertility of frozen-thawed semen in the chicken. Poultry Sci **64,** 1801–1803.

Bentley LG, Ansah GA, Buckland RB, 1984: Seminal plasma proteins of a line of chickens selected for fertility of frozen-thawed semen and the control line. Poultry Sci **63,** 1444–1445.

Fraser L, Pareek CS, Strzezek J, 2008: Identification of amplified fragment length polymorphism markers associated with freezability of boar semen-a preliminary study. Med Wet **64,** 646–649.

Guthrie HD, Welch GR, 2005: Impact of storage prior to cryopreservation on plasma membrane function and fertility of boar sperm. Theriogenology **63,** 396–410.

Hernandez M, Roca J, Ballester J, Vazquez JM, Martinez EA, Johannsson A, Saravia F, Rodriguez-Martinez H, 2006: Differences in SCSA outcome among boars with different sperm freezability. Int J Androl **29,** 583–591.

Hernandez M, Roca J, Calvete JJ, Sanz L, Muino-Blanco T, Cebrian-Perez JA, Vazquez JM, Martinez EA, 2007a: Cryosurvival and in vitro fertilizing capacity postthaw is improved when boar spermatozoa are frozen in the presence of seminal plasma from good freezer boars. J Androl **28,** 689–697.

Hernandez M, Roca J, Gil MA, Vazquez JM, Martinez EA, 2007b: Adjustments on the cryopreservation conditions reduce the incidence of boar ejaculates with poor semen freezability. Theriogenology **67,** 1436–1445.

Holt WV, Medrano A, Thurston LM, Watson PF, 2005: The significance of cooling rates and animal variability for boar sperm cryopreservation: insights from the cryomicroscope. Theriogenology **63,** 370–382.

Larsson K, Einarsson S, 1976: Influence of boars on the relationship between fertility and post thawing sperm quality of deep frozen boar spermatozoa. Acta Vet Scand **17,** 74–82.

Medrano A, Watson PF, Holt WV, 2002: Importance of cooling rate and animal variability for boar sperm cryopreservation: insights from the cryomicroscope. Reproduction **123,** 315–322.

Mitchell RL, Buckland RB, Kennedy BW, 1977: Heritability of frozen and fresh chicken semen and the relationship between the fertility of frozen and fresh semen. Poultry Sci **56,** 1168–1177.

Pizzi F, Gliozzi TM, Cerolini S, Maldjian A, Zaniboni L, Parodi L, Gandini G, 2005: Semen quality of Italian local pig breeds. Ital J Anim Sci **4,** 482–484.

Roca J, Hernandez M, Carvajal G, Vazquez JM, Martinez EA, 2006: Factors influencing boar sperm cryosurvival. J Anim Sci **84,** 2692–2699.

Safranski TJ, 2008: Genetic selection of boars. Theriogenology **70,** 1310–1316.

Scott TA, Buckland RB, Kennedy BW, 1980: The effect of selection for fertility of frozen-thawed semen on spermatozoa oxygen uptake, motility and concentration, and ejaculate volume in the chicken. Theriogenology **14,** 281–298.

Thurston LM, Siggins K, Mileham AJ, Watson PF, Holt WV, 2002: Identification of amplified restriction length polymorphism markers linked to genes controlling boar sperm viability following cryopreservation. Biol Reprod **66,** 545–554.

Waterhouse KE, Hofmo PO, Tverdal A, Miller RR Jr, 2006: Within and between breed differences in freezing tolerance and plasma membrane fatty acid composition of boar sperm. Reproduction **131,** 887–894.

Woelders H, Matthijs A, Den Besten M, 1995: Boar variation in ''freezability'' of the semen. Reprod Dom Anim **31,** 153–159.

Yousif YF, Ansah GA, Buckland RB, 1984: Effect of selection for fertility of frozen-thawed semen in chickens on the fertility of fresh and stored semen. Poultry Sci **63,** 1475–1480.

Submitted: 1 March 2011; Accepted: 12 June 2011

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